J. Asia-Pacific Entomol. 4 (2) : 201～207 (2001)
www.entomology.or.kr

The Biotypes, Wing-forms and the Immigration of Brown Planthopper, Nilaparvata lugens Stål, in Zhejiang Province, China

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Abstract The virulence change (biotype), wing form development and immigration of brown planthopper (BPH) *Nilaparvata lugens* Stål, population in Zhejiang province was studied as compared to these BPH populations collected from Guangxi and Yunnan province, China and Philippines. The results indicate that the Zhejiang population could successfully infest variety IR26 and Mudgo after 1989, and ASD7 and IR36 after 1998, showing the characters of BPH biotype 2 and biotypes 3. The BPH tropical populations such as Guangxi population, however, adapted the resistant varieties IR26, Mudgo and ASD7 earlier. In 1997, the nymphal survival indices of Guangxi and Yunnan populations on Rathu Heenati (*Bph3*) were up to 70.9 and 66.7 respectively, higher than Zhejiang population. The Zhejiang populations show low brachupterous rates and have a negative correlation with nymphal density, belonging to the temperate type. The Philippine populations are almost brachypterous, however, the brachypterous rates of BPH males raise with the increase of nymphal density. The Guangxi population has a high brachypterous rate, being similar to the tropical type. On the other hand, tests showed that the lowest survival rate, longer duration of nymphs and the lightest weight of brachypterous female occurred in the 2nd generation of BPH after continuously feeding on resistant variety. Physiologically, the highest activities of superoxide dismutase (SOD) and catalase (CAT) were detected in the second generation of the BPH feeding on resistant rice variety.

Key Words Virulence, wing form, immigration, *Nilaparvata lugens* Stål, Enzyme SOD and CAT

Introduction

The brown planthopper (BPH), *Nilaparvata lugens* Stal, characterized by its monsoon migration and rstrategy life pattern in East Asia (Sogawa, 1995; Planka, 1970; Wu *et al.*, 1983), is one of the most important insect pests on rice in China. It usually reproduces through the year in subtropical and temperate regions south of about 220 N latitude (Cheng *et al.*, 1979), and migrates from Mekong Delta in Indo-China to Guangxi Province in southern China from May to June, then to Yangtze Delta located in East China during June-August (Wu *et al.*, 1997). Yield loss of at least one million tons was caused directly by its feeding damage every year. Especially in 1991, more than 2.5 million tons were lost because of the widely severe outbreak of BPH in China (Li *et al.*, 1996).

At the beginning of the 1970's, the resistant rice variety IR26 (bearing gene *Bph1*) bred in International Rice Research Institute (IRRI) was released widely in some Asian countries for suppressing the BPH population. However, the BPH population adapted IR26 to be a new biotype only 3 years later (IRRI 1976; Saxena and Sogawa 1977). It was reported that the biotype of BPH in Guangxi and Hainan located in southern China had shifted from biotype 1 to 2 since 1987 (Li *et al.*, 1991; Tao *et al.*, 1992) and the field population of BPH in Zhejiang was dominated by biotype 2 in 1989 (Yu *et al.*, 1991; Tao *et al.*, 1992). The breeding and releasing of resistant rice varieties were obviously threatened by the development of new BPH biotypes in Asia.

This study was carried out to clarify the virulence shifting of BPH populations in Zhejiang province, china, as compared these BPH collected from other locations, on various resistant varieties. The shifting mechanism of BPH on resistant varieties was analyzed. The wing-form of BPH biotypes at different nymphal densities was studied to report the immigration of BPH. All tests were conducted in Hangzhou, Zhejiang Province.

Materials and Methods

Insect collection and rearing

The various geographic populations of BPH were

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(Received April 6, 2001; Accepted August 22, 2001)

collected in the fields of Guangxi and Yunnan Provinces in southern China and Zhejiang Province in Yangtze Delta during June-August and were reared on the susceptible rice variety TN1 before doing experiments. The BPH immigrants were collected using light trap, and maintained in cages feeding with TN 1 plants in male and female, respectively.

Virulence determination

A modified method developed by Wu *et al.* (1990) was used for evaluating the virulence of BPH populations collected from different localities. The seeds of rice varieties such as TN1, Mudgo, IR26, ASD7, IR36, Rathu Heenati (RH) and Ptb33 were sown in a 60cm 45cm 10cm seedbox, 10plants per row for every tested varieties and 3 replications were set up. In 3-leaf stage, about 5 to 6 1st or 2nd instar nymphs per seedling were used for infestation. Damage ratings were recorded, when the 90% susceptible check TN1 seedlings were dead; grades for every tested varieties were evaluated using the Standard Evaluation System for Rice (SES) (IRRI, 1980). The virulence of various BPH populations was determined according to the grades in every tested variety.

Nymphal survival rate

The seedlings of all tested varieties were transplanted into the field in 4-leaf stage. During rice tillering stage, ten 4-5th instar nymphs were introduced on each plant enclosed with a mylar cage (diam 8cm, height 60cm) covered with nylon mesh. The numbers of remaining BPH were counted after 10 days of the plant infestation. The nymphal survival index (NSI) was calculated as follows : NSI= [(survival rate (%) of BPH on a tested variety)/(survival rate (%) of BPH on TN1)] $\times$ 100

Oviposition and reproduction of BPH immigrants

A total of 200 immigrated females was collected from light trap and maintained on TN1 plants in mylar cages (Dia. 3cm, Height 30) individually. All plants were replaced daily to dissect and count the eggs laid by each female (100 replications), and in another test, the ovary of female was dissected and eggs in ovary were counted. The field populations were divided into two groups, the females were mated in one group, and other females remained unmated with males. 100 replications were tested in each group. The pre-oviposition and oviposition duration, fecundity, and hatchibility were tested and calculated.

Development of wing form

The 1st instar BPH nymphs of various biotypes were individually introduced into mylar cages with rice plant TN1. The various nymphal densities were arranged as 1, 5, 10, 20, 40 nymphs per plant, and all plants were refreshed 3 days intervals. The percentage of brachypterous females or males were counted and calculated in each treatment. All tests were carried out in the constant cabinets of temperature $26\pm1^{\circ}C$, L:D=16:8 and humidity 65-85%.

Biotype shifting of BPH on resistant varieties

The 1st instar BPH nymphs were individually introduced into mylar cages containing a plant of different resistant variety, and maintained in the constant cabinets. The tests were replicated 120 times for each variety, and the development of nymphs was checked and recorded every day. The newly emerged females were weighed using balance, and kept in pair on the same variety for further generation.

Meanwhile, the 10 newly hatched adults feeding on resistant variety IR26 were homogenized in 4ml of Tris buffer (pH7.2, 0.1mol/L) and centrifuged for 5 minutes at 15,000g/min. The supernatant was stored in deep-frozen refrigerator for further analysis.
Enzyme SOD, CAT and POD at different generation BPH were analyzed as the methods illustrated by Li (1994).

Results

Virulence shifting of BPH populations on rice varieties bearing different resistance genes during 1981-2000

The virulence of BPH field population was monitored using various rice varieties bearing different resistance genes during 1981-2000 (Table 1). The results shown that less 50% of BPH can survive for ten days on all tested resistance varieties bearing *bph1, bph2* and *Bph3* gens as compared susceptible rice variety TN1 (check variety without resistance gene to BPH) before 1989. The survival index suddenly increased >50% on rice varieties IR26 and Mudge bearing resistance gene Bph1 after 1989 and 1990 respectively. It indicated that the majority of BPH immigrants into Zhejiang province were BPH biotype 2. The BPH immigrants can infest rice varieties ASD7 and IR36 bearing resistance gene *bph 2* after 1998 and 1999, respectively. In the recent years, almost half of BPH immigrants

Table 1. The nymphal survival index (NSI) of BPH population collected in Zhejiang province on different rice varieties with various resistance genes during 1981-2000

Year	Nymphal survival index (NSI) of *N. Lugens* on various rice varieties bearing resistant genes						
	TN1 None(CK)	Mudgo *Bph1*+?	IR26 *Bph1*	ASD7 *Bph2*	IR36 *Bph2*+?	R. H *Bph3*	Pth33 *Bph3*
1981	100	15.8	24.3	*	18.4	*	*
1982	100	28.2	16.8	15.8	7.1	32.0	25.4
1983	100	4.7	3.7	17.2	5.6	11.3	0.5
1984	100	32.3	35.5	6.5	19.4	38.7	9.7
1986	100	23.1	46.2	*	2.6	*	2.6
1987	100	9.2	21.6	*	20.2	23.1	15.4
1988	100	*	10.2	*	3.4	*	*
1989	100	41.2	80.9	44.1	14.7	0	9.9
1990	100	69.6	108.5	*	28.7	*	3.0
1991	100	52.3	75.4	48.5	18.5	*	*
1992	100	78.2	87.5	30.5	9.4	*	*
1993	100	87.2	88.8	40.4	31.3	3.0	8.3
1994	100	64.3	89.3	34.3	35.7	25.0	7.1
1995	100	52.7	100	23.6	20.0	25.5	7.8
1996	100	58.8	72.8	36.6	14.4	36.6	*
1997	100	*	76.9	42.7	31.7	48.8	*
1998	100	51.0	94.3	64.1	35.8	45.2	*
1999	100	*	126.8	63.4	56.1	*	*
2000		*	53.4	65.1	52.5	47.3	3.4

can survive on resistant variety *bph3* with gene *bph3*, however, there were low survival rates on variety Ptb 33 (the same resistance gene *bph3*) as compared to the susceptible check TN1. The yearly monitoring proved that the virulence of BPH populations in Zhejiang improved gradually due to the annual immigration of BPH, and caused the infestation on rice varieties bearing various resistance genes after the BPH population was dominated by a specific virulent immigrants.

The damaged grades (Table 2) of rice seedlings caused by BPH population collected in Zhejiang province were investigated on resistant varieties IR26 and Mudgo while 90% seedlings of susceptible check TN1 were killed. The BPH populations caused the similar damage on variety IR26 to susceptible check TN1 during 1994-2000, and had less infestation on variety Mudgo probably due to the expression of minor gene. The damage grades of Zhejiang population on variety ASD7, IR36 and Rathu Heenati were significantly lower as compared to the susceptible check, however, more damage was caused on variety Rathu Heenati after 1999. Even the virulence of BPH immi-

Table 2. The damage rates of various rice seedlings infested with BPH population collected in Zhejiang province, China

Rice variety	Damage rates on various resistant varieties infested with BPH						
	1994	1995	1996	1997	1998	1999	2000
TN1 (susceptible control)	9	9	9	9	9	9	9
IR26	9	9	9	9	7	9	9
Mudgo	5	3	3	9	5	5	5
ASD7	1	3	1	5	1	0	3
IR36	0	3	0	3	1	3	3
Rathu Heenati (RH)	1	0	1	3	/	5	5
Pth33	0	1	0	0	1	0	0

Table 3. The NSI of various geographic populations on resistant varieties in 1997

Rice variety	NSI[a] of various BPH populations		
	1994	1995	1996
TN1	100	100	100
IR26	76.8	98.7	92.8
ASD7	42.7	63.3	73.9
IR36	31.7	36.7	56.5
Rathu Heenati	48.8	70.9	66.7

[a]Nymphal survival index.

grants on resistance varieties promoted yearly, the rice damage caused by BPH population was less because of differences of rice compensation.

The results in Table 3 shown that the virulence of BPH populations collected from Guangxi and Yunnan province were higher than that collected from Zhejiang, on all tested resistant varieties in 1997. It imply that the BPH populations in tropical or subtropical areas shift their virulence earlier than BPH populations in temperate area such as Zhejiang province.

The wing forms of BPH populations immigrated into Zhejiang province and collected from different locations

The BPH immigrants collected from light traps in Zhejiang province could develop their ovary and laid similar number of eggs on rice plants as compared to the mated and unmated BPH females collected from rice fields. However, no eggs laid by immigrants hatched and was the same as the eggs laid by unmated BPH females (Table 4). It indicated that the immigrants into Zhejiang did not mated before landing.

The results in Table 5 shown that brachypterous rates of BPH populations collected from Hainan and Guangxi province (tropical areas) were higher than those of BPH populations collected from Zhejiang province (temperate and immigrated area). The percentage of brachypterous females in Hainan and Zhejiang province decreased with the increase of nymphal densities, however, there was no significant correlation between brachypterous rates of female and nymphal densities in Guangxi population. On the other hand, the much lower percentage of brachypterous males was developed at the low density (1 head per plant) for Hainan and Guangxi populations.

The 3 BPH biotypes collected in the Philippines had high brachypterous rates at different nymphal densities, there were almost 100% of brachypterous females at any tested densities. The brachypterous rates of BPH males increased with the increase of nymphal densities, showing the same characteristics of wing-form development with Guangxi BPH population (Tables 5 and 6). It also indicated that there was no obvious difference of wing forms at various nymphal densities between different biotypes.

The shifting and physiological mechanism of BPH biotypes feeding on various resistance varieties

The adaptation test of BPH immigrated population on resistance varieties in the different progenies was shown in Table 7. The nymphal survival rates of BPH increased gradually after continuously feeding on resistant varieties IR26, Mudgo and ASD7. However, the 1st progeny of BPH had the lowest survival rate and adult weight after feeding on variety IR26, Mudgo and ASD7 for one complete generation. After feeding on these varieties for 3 generations, the nymphal survival rates and adult weight of BPH were similar to BPH feeding on susceptible check TN1, indicating the adaptation of BPH immigrants to these resistance varieties.

The activities of enzyme SOD, CAT and POD in the brachypterous females feeding on resistant variety IR26 were analyzed at the various generations (Table 8). The activities of SOD and CAT were higher in the 1st progeny of BPH than other generations feeding on IR26, it imply that the second generation on

Table 4. The oviposition, fecundity and hatchibility of brown planthopper immigrants in Zhejiang province, china

BPH	Sex	Fecundity, pre- and oviposition duration, hatchibility of BPH					
		Eggs in ovary per female	Pre-oviposition duration (d)	Oviposition duration (d)	Fecundity (No./female)	% of females laid eggs	Hatchibility (%)
Immigrants	100F	71.3b	5.7b	9.8a	79.6a	69.4b	0
Mated female	100F	48.8b	6.4b	9.9a	48.2b	40.0b	0
Un-mated female	100F +100M	105.4a	3.7a	9.3a	87.4a	85.7a	91.5

Means in a column followed by same letter are not significantly different ($P<0.05$).

Table 5. The correlation of brachypterous rates and nymphal densities of BPH collected from different locations in China

Geographic populations	Sex	Brachypterous percentage of BPH at different nymphal densities					r[a]
		1 nymph/tiller	5 nymphs/tiller	10 nymphs/tiller	20 nymphs/tiller	40 nymphs/tiller	
Linshui, Hainan	F	75.0	54.5	66.7	37.1	23.8	-0.9157*
	M	0	33.3	13.3	9.3	18.0	0.1047
Longzhou,Guangxi	F	66.7	100	76.5	78.9	71.1	-0.2838
	M	0	21.4	20.0	40.0	34.1	0.7438
Wenzhou, Zhejiang	F	60.0	42.8	56.3	31.2	9.9	-0.9351*
	M	0	0	7.1	5.0	1.4	0.1094
Longyou, Zhejiang	F	20.0	20.0	12.1	5.9	4.9	-0.88101*
	M	0	0	0	0	0	
Hangzhou,Zhejiang	F	66.7	53.3	40.9	37.9	23.9	-0.9195*
	M	14.2	10.0	15.8	3.8	7.5	-0.5958

[a] Correlation of brachypterous percent and density.

resistant variety is very important period for BPH to adapt physiologically new resistant variety.

Discussion

It is clear that the Zhejiang BPH population has been dominated by biotype 2 since 1989 and dominated by biotype 2 and 3 after 1998. The shift of biotype 2 in Zhejiang BPH population, which was 2 years later than Guangxi BPH population (Zhang *et al.*, 1990) and 13-14 years later than tropical countries (IRRI, 1976), it imply that the shifting order of biotype occurred geographically from south to north. It may be explained as follows: 1) more individuals of biotype 2 in a migratory population, which have weaker life vigor than biotype 1, were eliminated and only a little stronger individuals immigrated successfully into the destinations (Saxena and Barrion, 1985; Wu *et al.*, 1990). The higher tolerance to starvation found in biotype 1 than biotype 2 (Lu and Yu, 1999) was a sound example. The longer of distance BPH population migrated, the lower of proportion biotype 2 occupied in BPH population. It was more difficult to become the dominant for biotye 2 in an immigrated population than emigrated area. 2) In temperate areas, the shifting of biotypes is usually interrupted by the coming of winter, so the proportion of biotype 2 in BPH population could not be accumulated year by year, obviously depending on the virulence of BPH immigrants from the source regions. 3) Shanyu no.6 variety carrying Bph1, released widely in the early period of 1980's in China, was used quite later than IR26 grown in tropical countries, and the proportion of area planted rice varieties resistant to BPH in China was much less than that in other countries in Southeast Asia. The successful infestation of BPH immigrants in Zhejaing on variety IR36 is the another example to indicate that the Zhejiang BPH population had been dominated by the BPH individuals capable of feeding on these varieties bearing resistance gene *bph2* after 1998.

New changes of virulence to resistant rice varieties have been found in Guangxi and Yunnan BPH populations in 1997. The rice varieties IR26, Mudgo, ASD7 and R.H. bearing various resistant genes have been adapted, and the virulence characteristics of Guangxi and Yunnan populations were similar to those of "Goimbatore" type in India (IRRI, 1982). It was proved that the virulence of Zhejiang BPH population shifted again after 1998 on resistant variety

Table 6. The brachypterous rates of different BPH biotypes at various nymphal densities (IRRI)

Variety	TN1		Mudgo		ASD7	
Biotypes	Biotype 1		Biotype 2		Biotype 3	
Density	Female	Male	Female	Male	Female	Male
1	100	25.0±21.0	100	16.6±20.3	100	0
5	100	53.8±22.1	100	50.0±23.8	100	68.4±25.4
10	100	68.0±12.3	100	62.1±21.1	100	81.3±24.0
20	100	75.0±15.6	100	77.1±13.0	100	91.1± 8.1
40	100	86.8±11.2	98.1±1.8	77.4± 3.8	100	96.4± 3.0

Table 7. Survival, nymphal duration, body weight and population growth of brown planthopper in different progenies on various resistant varieties

	IR26					Mudgo				ASD7				TN1
Parameter	1st	2nd	3rd	4th	5th	1st	2nd	3rd	9th	1st	2nd	3rd	12th	(CK)
Survival rate (%)	43.58	9.17	74.49	77.23	86.72	27.5	2.52	65.7	63.79	28.32	1.87	5.38	65.0	76.92
Nymphal duration (d)	12.67	18.11	11.56	12.42	12.12	14.01	17.1	13.25	13.61	11.92	12.57	13.34	13.82	13.83
Body weight (mg/head)	1.98	1.67	2.79	2.57	2.68	1.98	1.50	2.59	2.62	1.88	1.50	2.23	2.75	2.92
Population growth				81.58	91.28				91.85				93.45	105.13

Table 8. The activities of superoxide dismutase (SOD), catalase (CAT) and (POD) of various BPH progenies after feeding on resistant variety IR26

Enzymes	Activities of various enzymes of BPH progenies after feeding on resistant variety IR26				
	48 h	Generation			
		1st	2nd	3rd	5th
SOD (Inhibition %)	17.2	17.9	25.5	19.5	2.9
CAT (ml H_2O_2)	0.05	0.15	0.35	0.25	0.10
POD (OD Value)	0.02	0.013	0.017	0.035	0.025

ASD7 and IR36. It may also imply that most resistant rice varieties bearing *Bph1* and *bph2* genes would be threatened and damaged by the new change of virulence in Zhejiang Province. Our test further confirmed that it only took 3 generations for BPH to adapt a new resistant variety after a continuous feeding. It was reported that BPH could completed 4-5 generations in Zhejiang province (Du, 1991), it may caused the change of BPH virulence after immigration in the widely released area of new resistant variety. However, the winter interruption of the BPH life cycle and the rice compensation will delay these changes. In fact, the change of virulence in Zhejiang province depended on the virulence of BPH immigrants. The adaptation of BPH to resistant variety, and the occurrence of biotype 2 was the result of growing variety IR_{26} widely in tropical countries.

The development of wing form in BPH population from Zhejiang were different from these BPH populations collected from tropical and subtropical areas in China. The dissection of BPH immigrants shown their unmated before landing.

Acknowledgements We express our sincere thanks to Mr. Jian Tang at CNRRI and Mr. Fenkuang Huang at Guangxi Academy of Agricultural Sciences for their kind help in collecting Yunnan and Guangxi BPH populations.

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